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TEST-RETEST RELIABILTY OF EXERCISE-INDUCED HYPOALGESIA AFTER AEROBIC EXERCISE

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ABSTRACT

Objective: Exercise increases pressure pain thresholds (PPTs) at exercising and non-exercising muscles known as exercise-induced hypoalgesia (EIH). No studies have investigated test-retest-reliability of change in PPTs after aerobic exercise. Primary objectives were to compare the effect on PPTs after an incremental bicycling exercise compared with quiet rest, and investigate relative and absolute test-retest reliability of the test stimulus (PPT) and the absolute and relative EIH response at exercising and non-exercising muscles.

Setting: Laboratory

Methods: In two sessions, PPTs at the quadriceps and trapezius muscles were assessed before and after 15 min quiet rest and 15 min bicycling in 34 healthy subjects. Habitual physical activity was assessed by the International Physical Activity Questionnaire (IPAQ).

Results: Bicycling increased PPTs at exercising and non-exercising muscles in both sessions ($P<0.05$). The magnitude of the EIH response at the exercising muscle was however larger in second compared with first session ($P<0.015$). PPTs showed excellent ($ICC\geq0.84$) within-session and between-sessions test-retest reliability. The EIH response at exercising and non-exercising muscles demonstrated fair ($ICC=0.45$) between-sessions relative test-retest reliability, but agreement in EIH responders between sessions was not significant (Quadriceps: $\kappa=0.24$, $P=0.15$; Trapezius: $\kappa=0.01$, $P=0.97$). Positive correlations between the IPAQ score and PPTs were found (Quadriceps: $r=0.44$, $P=0.009$; Trapezius: $r=0.31$, $P=0.07$) before exercise. No significant association was found between IPAQ and EIH.

Conclusions: Incremental bicycling exercise increased PPTs with fair relative and absolute reliability of the EIH response. These data might have impact on future studies investigating EIH and for clinicians designing exercise programs for pain relief.

Key words: exercise, pressure pain thresholds, pain sensitivity, hypoalgesia

1. INTRODUCTION

The effect of exercise on pain perception in humans is of growing interest (1). Several experimental studies have demonstrated a robust decrease in the pain sensitivity at exercising and non-exercising muscles during and following different exercise protocols in healthy subjects (2). This phenomenon has been referred to as exercise-induced hypoalgesia (EIH) (3). Several protocols assessing the effect of exercise have been described. Aerobic exercise conditions (e.g. bicycling) produce a hypoalgesic response when performed at moderate to high intensities (4) whereas isometric exercise conditions (i.e. a muscle contraction without joint movement) produce EIH at both low and high intensities (5). Experimentally, EIH can be assessed using thermal, electrical or pressure stimuli as test stimulus before and during or after the exercise condition. The EIH response is typically calculated as the absolute or relative difference in the test stimulus during or after the exercise condition compared with the test stimulus before the exercise condition. To date, the mechanisms underlying EIH is not clear but recent studies have indicated that mechanisms relevant to descending pain control assessed by conditioned pain modulation (CPM) may contribute to the EIH response, due to the painful experience during exercise (6, 7).

Although EIH has been investigated extensively in healthy volunteers, at present, there is no consensus whether a certain EIH protocol is preferable over others. To explore clinical applicability of EIH after an exercise condition, an analysis of the test-retest reliability in healthy subjects is an essential prerequisite. No studies have analyzed the test-retest-reliability of the commonly used method with pressure pain thresholds (PPTs) as the test stimulus and bicycling as the exercise condition (4), although this protocol seems to provide clinically relevant information on the capacity of endogenous pain modulation in patients with chronic pain (8), and a predictor of treatment response (9). Since exercise is commonly used as part of treatment programs for chronic pain further knowledge on the between-session reliability is important.

In addition to acute exercise, habitual physical activity has been linked to alterations in pain perception, and athletes have decreased pain sensitivity compared with normally active controls (10). Few studies in healthy subjects have examined the relationship between habitual physical activity and EIH. Interestingly impaired EIH is often found in chronic pain patients (2) in whom also physical activity is often reduced (11).

This study aimed to 1) compare the effect on PPTs after an incremental bicycling exercise compared with quiet rest in healthy subjects, 2) investigate test-retest reliability of the test stimulus (PPT) and the EIH response at exercising and non-exercising muscles, and 3) investigate the influence of habitual physical activity on PPTs and EIH. It was hypothesised that 1) the bicycling exercise would produce an increase in PPTs at exercising and non-exercising muscles compared with quiet rest, 2) PPTs and EIH would demonstrate acceptable test-retest reliability, and 3) greater habitual physical activity would be associated with higher PPTs and EIH.

2. MATERIALS AND METHODS

2.1 Subjects

Thirty-four healthy subjects (mean age of 25.8 ± 3.4 years; [range 21–39 years]; average body mass index (BMI) 25.1 ± 3.8 kg/m^2 [range 18.4-34.7]; 5 left-handed; 13 females) were included in this study that was conducted in accordance with the Declaration of Helsinki, approved by the local ethical committee (S-20160189) and all subjects provided written informed consent. The subjects were recruited by advertisement at the local university college, and through social media. None of the included subjects suffered from neurological, psychological, cardiovascular diseases, had any pain or used any pain medication during the weeks prior to participation. All subjects were asked to refrain from physical exercises, coffee and nicotine on the days of participation. An a priori power analysis determined that with a power of 0.80, alpha $\leq$ 0.05, and a paired t-test, 34 participants were required to detect a difference in EIH with a moderate effect size (d = 0.50) between session 1 and session 2.

2.2 Procedure

Subjects participated in two identical sessions at the same time of the day and separated by 1 week (Fig. 1). This time frame was chosen to minimize potential carry-over effects from the pain sensitivity assessments and exertion after physical exercise between sessions as well as avoiding extensive changes in physical fitness level within subjects. In the beginning of session 1, all subjects completed the short-version International Physical Activity Questionnaire (IPAQ), and were verbally introduced to the procedures and familiarized to assessment of PPT on the non-dominant thigh, which was not used for further assessments. In each session, PPTs were initially recorded from the dominant thigh and the non-dominant shoulder. In addition, all subjects performed a 15 min quiet rest condition and a 15 min bicycling exercise in each session. PPTs were assessed before and immediately after the quiet rest and exercise.

2.3 Pressure pain threshold assessment

A handheld pressure algometer (Somedic Sales AB, Sweden) with a stimulation area of 1 cm^2 was used to assess PPTs at two assessment sites. Site one was located in the middle of the dominant quadriceps muscle (exercising muscle), fifteen centimetres proximal to the base of patella. Site two was located in the non-dominant upper trapezius muscle (non-exercising muscle), ten centimetres from the acromion in direct line with the neck. The pressure from the algometer was increased at approximately 30 kPa/s and the first time the pressure was perceived as minimal pain, the subject pressed a button and the pressure intensity defined the PPT. Two assessments were completed for each site and the average was used for analysis.

2.4 Quiet rest and bicycling conditions

In the quiet rest condition, subjects were instructed to relax in a seated position in a comfortable armchair for 15 min in a 21 degrees Celsius temperate and undisturbed room.

Subjects performed 15 min incremental bicycling exercise conditions. The seat post of the stationary cycle (Ergomedic 928E, Monark Exercise AB, Vansbro, Sweden) was adjusted so that the subject had five degree bend at the knee during the bottom phase of the pedal stroke. A heart rate monitor (Monark Heart Rate Monitor) was strapped around the subject's chest. Just before the exercise condition the subject was instructed to rate pain intensity in the legs on a 0-10 numerical rating scale (NRS), with 0 defined as "no pain" and 10 "as worst imaginable pain", and rating of perceived exertion (RPE) on Borg's 6-20 scale, with 6 defined as "no exertion at all" and 20 as "maximal exertion". Heart rate was also assessed. The start intensity was set to 20 Watts and resistance was then increased by 20 Watts per min until a RPE of 16 was achieved where after the subject continued bicycling at that intensity for the remaining time. Subjects were instructed to maintain a pedal rate as close to seventy rounds per min (RPM) as possible throughout the 15 min bicycling exercise. Heart rate was monitored constantly, pain intensity in the legs and RPE were assessed after 2, 3, 6, 9, 12 and 15 min, and maximum intensity in Watts was recorded. The exercise condition in session two was identical in terms of increase in Watts despite potential differences in heart rate, pain intensity or RPE. This was chosen to ensure that the objective intensity of the exercise sessions did not differ between the two sessions.

2.5 International Physical Activity Questionnaire

The short version International Physical Activity Questionnaire (IPAQ) was used to assess frequency and duration of vigorous, moderate and light physical activity undertaken during the last seven days. In addition, time spent with sedentary activity was also assessed with the questionnaire. For each of the domain the metabolic equivalent (MET) per minutes was calculated and the domain were summed for a total MET score (12).

2.7 Statistics

Results are presented as mean and standard deviation (SD) in the text and as mean and standard error of the mean (SEM) in figures. The distribution of PPTs, peak NRS scores and heart rate did not deviate significantly from normality (Shapiro-Wilks test: $P > 0.15$). The effect of sessions and gender on baseline (pre-rest) PPTs was analyzed with a mixed-model analysis of variances (ANOVAs) with *session* (session 1 and session 2) and *assessment sit*e (quadriceps and trapezius) as within subject factor and *gende*r as between subject factor. The effects of exercise and rest on PPTs were analyzed with a mixed-model ANOVAs with *session* (session 1 and session 2), *condition* (exercise and rest), *assessment site* (quadriceps and trapezius), and *time* (before and after) as within subject factors and *gende*r as between subject factor. Furthermore, absolute (PPT-after exercise minus PPT-before exercise) and relative (percentage increase of the PPT-after versus the PPT-before) differences in PPTs after exercise was calculated (defined as EIH). Potential differences in absolute and relative EIH between women and men were investigated with independent t-tests. Changes in heart rate, RPE and NRS scores, during exercises were analyzed with ANOVAs with *session* (session 1 and session 2), and *time* (0, 2, 3, 6, 9, 12, and 15 min) as within subject factors and *gende*r as between subject factor. *P*-values less than 0.05 were considered significant. In case of significant factors or interactions in ANOVAs, Bonferroni corrected post-hoc tests were used to correct for multiple comparisons. Spearman correlational analyses were performed to determine possible associations between the total IPAQ score, baseline PPTs, and absolute and relative EIH.

For assessment of test-retest reliability of PPTs and the EIH response, the systematic error between sets of PPT assessments (within-session: PPTs before and after rest for each assessment site separately; between-session: baseline PPTs first and second session for each assessment site separately) and absolute and relative EIH responses between sessions were determined using repeated-measures ANOVA. Pearsons r and intraclass correlations (ICCs) based on a single rating, consistency, 2-way mixed effect model ($ICC_{3,1}$) were used to assess the reliability of the assessment of baseline PPTs and EIH responses to differentiate between subjects based on how their PPTs and EIH values ranked compared with the other subjects. An ICC above 0.75 was taken as excellent reliability, 0.40–0.75 was fair to good reliability, and less than 0.40 defined poor reliability (13). In addition, the within-subject test-retest reliability based on responders and non-responders were investigated. To classify subjects as EIH responders or non-responders in session 1 and session 2, respectively, the standard error of measurement (SEM) of repeated PPT assessments (before and after rest) in each session were estimated. SEM was calculated as the square root of the mean square error term in the repeated-measures ANOVA output (14). Subjects who had an increase in PPTs after exercise which was larger than the SEM was classified as EIH responders and subjects who did not have an increase in PPT which was larger than the SEM was classified as EIH non-responders. The frequency of EIH responders and non-responders was investigated and agreement between sessions was assessed with Cohen's kappa coefficient. Data were analyzed using SPSS Statistics, version 24 (IBM, Armonk, NY, USA).

3. RESULTS

3.1 Pain thresholds at baseline

PPTs had a tendency for being increased in men (quadriceps: 640 ± 141 kPa, trapezius: 349 ± 94 kPa) compared with women (quadriceps: 510 ± 220 kPa, trapezius: 299 ± 132 kPa; $F(1,32) = 3.60$, $P = 0.067$). A main effect of assessment site was found for baseline PPTs ($F(1,32) = 156.94$, $P < 0.001$) with post-hoc test showing that PPT at the quadriceps site was significantly higher compared with PPTs at the trapezius site in both men and women ($P < 0.001$). No significant differences in baseline PPTs were found between sessions ($F(1,32) = 2.52$, $P = 0.12$).

3.2 Comparison of exercise and quiet rest

The ANOVA of the PPTs demonstrated a significant interaction between conditions, assessment sites and time (Fig 2; $F(1,33) = 10.75$, $P = 0.002$), with post-hoc test showing increased PPTs after bicycling in session 1 and session 2 compared with before bicycling (Quadriceps mean increase: 18.5±17.2%; Trapezius mean increase: 12.7±27.3%, $P < 0.03$). In both sessions, the increase in PPT at the quadriceps was larger compared with PPT increase at the trapezius ($P < 0.001$). No significant differences in PPTs after rest were found ($P > 0.45$).

No significant gender differences in absolute or relative change in PPTs ($t(32) < 1.54$ $P > 0.13$) after exercise were found.

3.3 Comparison of exercise parameters between sessions

All subjects completed the exercise conditions during session 1 and session 2. Obviously the intensity of bicycling did not differ significantly between the two sessions as identical protocols were used, however a significant effect of gender was found (Fig. 3A; $F(1,32) = 20.27$, $P < 0.001$) with men reaching a higher exercise intensity than women. Exercise intensity increased significantly over time ($F(15,480) = 347.24$, $P < 0.001$) with post-hoc test showing higher intensity at each time point compared with the previous time point ($P < 0.001$) except between the last 5 assessments (11 min to 15 min).

The heart rate during exercise increased significantly over time (Fig. 3B; $F(6,192) = 787.37$, $P < 0.001$) with post-hoc test showing significantly higher heart rate at each time point compared with the previous time point ($P < 0.001$) except between the last two assessments (12 min and 15 min). Moreover, heart rate was higher during exercise in women compared with men ($F(1,32) = 7.70$, $P = 0.009$). No significant difference in heart rate between sessions were found ($F(1,32) = 3.09$, $P = 0.089$).

Ratings of perceived exertion was increased over time (Fig. 3C; $F(6,192) = 633.88$, $P < 0.001$) with post hoc test showing higher RPE at each time point compared with the previous time point ($P < 0.001$) except between the last two assessments (12 min and 15 min). Moreover, a significant difference was found between session 1 and session 2 ($F(1,32) = 26.45$, $P < 0.001$) with higher RPE during exercise in session 1 compared with session 2 ($P < 0.001$). In addition, RPE was significantly higher during exercise in women compared with men ($F(1,32) = 4.38$, $P = 0.044$).

The NRS ratings of pain intensity in the legs reported during bicycling increased over time (Fig. 3D; $F(6,192) = 85.38$, $P < 0.001$) with post hoc test showing higher pain scores at each time point compared with the previous time point ($P < 0.001$) except between the first two assessments (0 min and 2 min) and the last two assessments (12 min and 15 min. Moreover, a difference was found in reported pain scores between session 1 and session 2 ($F(1,32) = 9.03$, $P = 0.005$) with higher pain intensity during exercise in session 1 compared with session 2. No significant difference in pain intensity between men and women was found ($F(1,32) = 0.21$, $P = 0.65$).

3.4 Influence of habitual physical activity on baseline PPTs and EIH

A positive correlation was found between the total IPAQ score and baseline PPT at the quadriceps muscle ($r = 0.44$, $P = 0.009$) and almost between the total IPAQ score and baseline PPT at the trapezius ($r = 0.31$, $P = 0.07$). No significant associations were found between the total IPAQ score and absolute or relative change in PPTs after exercise ($P > 0.20$).

3.5 Test-retest reliability of PPTs and EIH

Within-sessions test-retest reliability of PPT at the quadriceps (Session 1: $F(1,33) = 0.83$, $P = 0.37$; Session 2: $F(1,33) = 1.22$, $P = 0.28$) and trapezius (Session 1: $F(1,33) = 0.52$, $P = 0.48$; Session 2: $F(1,33) = 2.32$, $P = 0.14$) muscles, respectively, showed no systematic errors between assessments, assessments were strongly correlated ($r \geq 0.87$), and ICCs were excellent with values ≥ 0.93 for both sites (Table 1).

Between-sessions test-retest reliability of PPT at the quadriceps ($F(1,33) = 4.12$, $P = 0.051$) and trapezius muscles ($F(1,33) = 0.12$, $P = 0.73$), respectively, showed no systematic errors ($F(1,33) < 4.124$, $P > 0.05$), which was also reflected in the 95 % CI of the mean differences, where zero lies within the interval. However, the difference in PPT at the quadriceps between sessions approached significance. Moreover, between sessions assessments were moderately correlated ($r \geq 0.72$), and ICCs were excellent with values ≥ 0.84 for both sites (Table 2).

Between-sessions test-retest reliability of EIH at the quadriceps (local EIH) and trapezius (remote EIH) sites were fair with ICCs of 0.45 and 0.46, respectively (Table 2). However, significant systematic error in EIH at the quadriceps between sessions was found ($F(1,33) = 6.575$, $P = 0.015$) with larger EIH response in session 2 compared with session 1. No systematic error in EIH at the trapezius site between sessions was found ($F(1,33) = 0.373$, $P = 0.546$). Moreover, correlations between the EIH responses at the quadriceps and trapezius between sessions were generally not significant.

3.6 Difference in PPTs after exercise considered to be real

The minimal differences needed between separate PPT assessments in a subject for the difference in the PPT to be considered real were 70 kPa and 70 kPa for quadriceps and 38 kPa and 42 kPa for trapezius in session 1 and session 2, respectively (Table 1). Sixteen and 19 subjects demonstrated increases in PPT at the quadriceps muscle larger than the SEM in session 1 and session 2, respectively with 11 subjects demonstrating larger increases in both sessions (Table 3; $\kappa = 0.24$ (95% CI, -0.08 to 0.56), $P = 0.15$). Fourteen and 12 subjects demonstrated increases in PPT at the trapezius muscle larger than the SEM at session 1 and session 2, respectively with 5 subjects demonstrating larger increases in both sessions ($\kappa = 0.007$ (95% CI, -0.33 to 0.34), $P = 0.97$).

4. DISCUSSION

This study is the first to investigate relative and absolute between-sessions test-retest reliability of exercise-induced hypoalgesia in healthy subjects. As hypothesised, the incremental bicycling exercise significantly increased PPTs at exercising and non-exercising muscles in both sessions. No significant differences in PPTs were found after quiet rest. Assessment of PPTs showed excellent within-session and between-sessions test-retest reliability. The EIH response at exercising and non-exercising muscles demonstrated fair between-sessions test-retest reliability, however, the magnitude of the EIH response at the exercising muscle was significantly larger in session 2 compared with session 1. Moreover, the agreement in EIH responders and non-responders between sessions was not significant. Finally, self-reported time spent on physical activity was positively associated with PPT at the quadriceps, but not with the EIH response.

4.1 The effect of exercise on pressure pain sensitivity

In agreement with previous research (15, 16) the current study demonstrated increases in manual PPTs at exercising and non-exercising muscles immediately after high intensity aerobic exercise. Moreover, the increase in PPT was higher at the exercising muscles compared with non-exercising muscles, which is in accordance with the findings from the only other study directly comparing local versus remote effects after aerobic exercise (4). These findings indicate that hypoalgesia after exercise is related to activation of systemic pain inhibitory mechanisms with widespread anti-nociceptive effects in concert with local or segmental pain inhibitory mechanisms. This finding could be related to the Gate Control Theory (17), where limb movement during exercise may excite large diameter afferent nerve fibers inhibiting nociceptive. Interestingly, in healthy subjects, passive movements induced local hypoalgesia compared with a control condition, indicating a potential role of joint movement or proprioception in EIH (18). Still, if this was a primary mechanism, low intensity aerobic exercise would be expected to produce a local EIH response in the exercising body parts, which is often not the case (4). A reduced EIH response after aerobic exercise has been related to increased widespread pressure pain sensitivity in patients with chronic musculoskeletal pain (8), and the difference in EIH between the quadriceps muscle and trapezius muscle could be related to different baseline pain sensitivity at the two assessment sites.

The most studied mechanism of the EIH response involves the endogenous opioid system, and, which may account for the multisegmental manifestations of EIH demonstrated in this study. Aerobic exercise results in an increased level of systemic β-endorphin (19, 20) although not directly correlated to the reduction in pain sensitivity (19, 21). Moreover, the effect on EIH after administration of naloxone, an opioid antagonist prior to aerobic exercise has demonstrated reduced EIH responses (22) although conflicting results have been demonstrated after aerobic exercise (21), and further research on the involvement of opiordergic mechanisms in EIH after aerobic exercise in warranted.

The multisegmental hypoalgesic response after exercise in this study may also be related to the experience of moderate intense pain during the exercise condition. To support the link between EIH and the 'pain inhibits pain' mechanisms, a study including 16 healthy women found that the hypoalgesic response after aerobic exercise was greater following painful exercise than non-painful exercise (6). In addition, previous studies in subjects with chronic pain have demonstrated an association between EIH and CPM (8, 23) indicating that subjects who demonstrate a greater ability to activate the descending inhibitory systems following a painful stimulus, report greater hypoalgesia following exercise. However, if 'pain inhibits pain' was the primary mechanism responsible for EIH in this study, greater EIH in session 1 would have been expected since more intense pain was reported during exercise in this session. In contrast, the EIH response was significantly larger in session 2. The experience of pain and discomfort during intense exercise is well recognized with an association between rating of muscle pain intensity and exercise work intensity (24), however it has previously been demonstrated that muscle pain intensity decreases over repeated bicycling trials as was also shown in the current study (25). As power output and cadence was kept constant between sessions it is possible that the reduced muscle pain rating in session 2 may be associated with the greater exercise-induced hypoalgesia in session 2 than 1.

No significant difference in EIH was found between women and men. Recent studies on the influence of gender on the EIH response have demonstrated mixed results. Some studies have shown comparable EIH responses in men and women (26-28), while other studies have shown larger EIH responses in women (29, 30). Limitations regarding the gender effects should be considered. Although different phases of the menstrual cycle do not appear to influence the magnitude of the EIH response in women (31), data were not collected in the current experiments on the use of contraceptives, status of menopause or menstrual cycle, which may affect the pain perception in the female participants (32). Despite a massive increase in the number of studies investigating EIH, no studies considered measurement error of the test stimulus as some of the change in test stimulus may be due to measurement error when quantifying the EIH response, and no information on EIH responders and non-responders considering these measurement errors have previously been reported. It is noteworthy, that approximately 30% of healthy subjects included in this study were classified as EIH non-responders, which warrants further studies investigating how exercise can be optimized to decrease pain sensitivity. Although, PPTs demonstrated excellent within-session reliability the variation between repeated assessments is approximately 10% which is somewhat similar to the change in PPTs after exercise demonstrated in this study. Assessment methods with less within-session variation could improve the reliability of exercise.

Finally, regular exercise has been linked with alterations in pain sensitivity and athletes have significantly higher pain tolerance (10), report less pain intensity during experimental pain (30), and demonstrate higher nociceptive withdrawal reflex threshold compared with normally active controls (33). These findings are supported by the results of the current study, where a significant association between time spent on physical activity reported on the IPAQ and PPT at the quadriceps was found, indicating that physical activity involving the lower extremity decreases the pain sensitivity in the lower limb. No significant association between time spent on physical activity and the EIH response was found which is in agreement with previous studies (28, 34).

4.2 Test-retest reliability of PPT and EIH

Within-session (rest) and between-session test-retest reliability for PPTs demonstrated excellent ICC values (>0.8) confirming previous studies reporting ICCs above 0.7 (35), suggesting that PPT is a reliable quantitative method to assess pain sensitivity in humans. In addition, the SEM was reported for PPTs at the quadriceps and trapezius muscles within a re-test period of 15 min an interval which might be more relevant in term of evaluation of EIH in future studies. It should be noted, that SEM for the PPTs were determined based on the rest condition which was always performed before the exercise condition. In case, the variability in PPTs decreases over time due to a training effect, the SEM could be overestimated.

Although multisegmental EIH was produced after the incremental aerobic exercise condition in both sessions, the between-session test-retest reliability for EIH only demonstrated fair reliability for both exercising and non-exercising muscles. Moreover, the agreement in EIH responders and non-responders between sessions was not significant. These findings could indicate that although aerobic exercise decreases pain sensitivity, considerable inter-individual difference in the magnitude of EIH between sessions exists. There may be several reasons for the discrepancies in EIH between days. Subjects may improve their EIH response simply due to training effects (e.g., performing the first test serves as practice for subsequent tests) or due to the experience of EIH during the first session which could induce expectations about EIH in the second session. This could potentially have great impact for the effect of exercise on pain sensitivity in patients with chronic pain as physical activity is often reduced (11) and where expectations about pain relief in response to exercise could be low. Interestingly, a smaller part of subjects were classified as EIH responders in session 1, but as EIH non-responders in session 2. Significant differences between sessions in self-reported ratings of exertion and leg pain intensity during exercise may also have influenced the within-subject test-retest reliability. Standardization of these factors in EIH protocols should be investigated in the future.

In patients with chronic pain, several studies have demonstrated impaired EIH compared with asymptomatic controls (36, 37), however the reliability of this response is unknown. However, the response may be expected to be even less reliable as pain patients presented higher variability in PPT compared with healthy participants (38) further increasing the SEM.

The current between-session EIH reliability show similar ICCs to what have been demonstrated for CPM responses. Although a recent systematic review on the test–retest reliability of CPM concluded that the intra-session reliability was good to excellent only 50% of the included studies found good to excellent between-session reliability (39). Strict standardization procedures and reduced bias induced by the person assessing pain sensitivity seems to increase reliability of CPM protocols (35, 40) and this should be considered in future EIH studies. The main limitations of this study were 1) the non-randomized order between quiet rest and exercise, 2) lack of blinding of the assessor, 3) the inclusion of only two test sessions as a plateau in EIH was not reached, and it could be hypothesized that the EIH response would become more stable over time due to stabilization of yet unknown physiological or psychological mechanisms affecting the EIH response, and 3) the lack of assessment of expectations about the effect of exercise on PPTs as the reliability measures of change in PPT could be reliability of such expectations and not necessarily the specific effects of exercise.

4.3 Conclusion

An incremental aerobic bicycling exercise consistently increased PPTs compared with a control condition in healthy subjects. Test-retest reliability of PPTs was excellent; however the relative and absolute reliability of the EIH response at exercising and non-exercising muscles were rather low. Subjects who spent more time on physical activity involving the lower extremities had higher PPT at the quadriceps, but time spent on physical activity was not associated with the EIH response. These data have may have an impact on future studies investigating EIH in subjects with and without pain and potentially for the practitioner which designs exercise programs for pain relief.

Future research is warranted to investigate 1) the reliability of different exercise protocols, 2) whether the EIH response can be further increased over time, and 3) the applicability of these findings in a clinical pain population.

References

1. Lima LV, Abner TSS, Sluka KA. Does exercise increase or decrease pain? Central mechanisms underlying these two phenomena. J Physiol 2017;595:4141-4150.

2. Naugle KM, Fillingim RB, Riley JL, 3rd. A meta-analytic review of the hypoalgesic effects of exercise. J Pain 2012;13:1139-50.

3. Koltyn KF. Exercise-induced hypoalgesia and intensity of exercise. Sports Med. 2002;32(8):477-87.

4. Vaegter HB, Handberg G, Graven-Nielsen T. Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. Pain 2014;155:158-67.

5. Hoeger Bement MK, Dicapo J, Rasiarmos R, Hunter SK. Dose response of isometric contractions on pain perception in healthy adults. Med Sci Sports Exerc 2008;40:1880-1889.

6. Ellingson LD, Koltyn KF, Kim JS, Cook DB. Does exercise induce hypoalgesia through conditioned pain modulation? Psychophysiology 2014;51:267-76.

7. Lemley KJ, Hunter SK, Hoeger Bement MK. Conditioned Pain Modulation Predicts Exercise-Induced Hypoalgesia in Healthy Adults. Med Sci Sports Exerc 2015;47:176-84.

8. Vaegter HB, Handberg G, Graven-Nielsen T. Hypoalgesia After Exercise and the Cold Pressor Test is Reduced in Chronic Musculoskeletal Pain Patients With High Pain Sensitivity. Clin J Pain 2016;32:58-69.

9. Vaegter HB, Handberg G, Emmeluth C, Graven-Nielsen T. Preoperative Hypoalgesia after Cold Pressor Test and Aerobic Exercise is Associated with Pain Relief Six Months after Total Knee Replacement. Clin J Pain 2017;33:475-484.

10. Tesarz J, Schuster AK, Hartmann M, Gerhardt A, Eich W. Pain perception in athletes compared to normally active controls: a systematic review with meta-analysis. Pain 2012;153:1253-62.

11. McBeth J, Nicholl BI, Cordingley L, Davies KA, Macfarlane GJ. Chronic widespread pain predicts physical inactivity: results from the prospective EPIFUND study. Eur J Pain 2010;14:972-9.

12. Ainsworth BE, Haskell WL, Hermann SD, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med Sci Sports Exerc 2011;43:1575-81.

13. Fleiss JL. The design and analysis of clinical experiments. Chichester (NY): John Wiley and Sons; 1986. 432 p.

14. Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. J Strength Cond Res 2005;19:231-40.

15. Koltyn KF, Garvin AW, Gardiner RL, Nelson TF. Perception of pain following aerobic exercise. Med Sci Sports Exerc 1996;28:1418-1421.

16. Naugle KM, Naugle KE, Fillingim RB, Samuels B, Riley JL, 3rd. Intensity Thresholds for Aerobic Exercise-Induced Hypoalgesia. Med Sci Sports Exerc 2014;46:817-825.

17. Melzack R, Wall PD. Pain mechanisms: a new theory. Science 1965;150:971-9.

18. Nielsen MM, Mortensen A, Sorensen JK, Simonsen O, Graven-Nielsen T. Reduction of experimental muscle pain by passive physiological movements. Man Ther 2009;14:101-109.

19. Janal MN, Colt EW, Clark WC, Glusman M. Pain sensitivity, mood and plasma endocrine levels in man following long-distance running: effects of naloxone. Pain 1984;19:13-25.

20. McMurray RG, Forsythe WA, Mar MH, Hardy CJ. Exercise intensity-related responses of beta-endorphin and catecholamines. Med Sci Sports Exerc 1987;19:570-4.

21. Droste C, Greenlee MW, Schreck M, Roskamm H. Experimental pain thresholds and plasma beta-endorphin levels during exercise. Med Sci Sports Exerc 1991;23:334-342.

22. Haier RJ, Quaid K, Mills JC. Naloxone alters pain perception after jogging. Psychiatry Res. 1981;5:231-232.

23. Fingleton C, Smart K, Doody C. Exercise-induced Hypoalgesia in People with Knee Osteoarthritis with Normal and Abnormal Conditioned Pain Modulation. Clin J Pain 2017;33:395-404.

24. Cook DB, O'Connor PJ, Eubanks SA, Smith JC, Lee M. Naturally occurring muscle pain during exercise: assessment and experimental evidence. Med Sci Sports Exerc 1997;29:999-1012.

25. Micalos PS, Marino FE, Kay D. Reduced muscle pain intensity rating during repeated cycling trials. J Sports Sci Med 2004;3:70-5.

26. Hoffman MD, Shepanski MA, Ruble SB, Valic Z, Buckwalter JB, Clifford PS. Intensity and duration threshold for aerobic exercise-induced analgesia to pressure pain. Arch Phys Med Rehabil 2004;85:1183-1187.

27. Kosek E, Lundberg L. Segmental and plurisegmental modulation of pressure pain thresholds during static muscle contractions in healthy individuals. Eur J Pain 2003;7:251-258.

28. Vaegter HB, Handberg G, Jorgensen MN, Kinly A, Graven-Nielsen T. Aerobic exercise and cold pressor test induce hypoalgesia in active and inactive men and women. Pain Med 2015;16:923-33.

29. Koltyn KF, Trine MR, Stegner AJ, Tobar DA. Effect of isometric exercise on pain perception and blood pressure in men and women. Med Sci Sports Exerc 2001;33:282-290.

30. Sternberg WF, Bokat C, Kass L, Alboyadjian A, Gracely RH. Sex-dependent components of the analgesia produced by athletic competition. J Pain 2001;2:65-74.

31. Hoeger Bement MK, Rasiarmos RL, DiCapo JM, et al. The role of the menstrual cycle phase in pain perception before and after an isometric fatiguing contraction. Eur J Appl Physiol 2009;106:105-112.

32. Riley JL, 3rd, Robinson ME, Wise EA, Price DD. A meta-analytic review of pain perception across the menstrual cycle. Pain 1999;81:225-35.

33. Guieu R, Blin O, Pouget J, Serratrice G. Nociceptive threshold and physical activity. Can J Neurol Sci 1992;19:69-71.

34. Øktedalen O, Solberg EE, Haugen AH, Opstad PK. The influence of physical and mental training on plasma beta-endorphin level and pain perception after intensive physical exercise. Stress and Health 2001;17:121-127.

35. Graven-Nielsen T, Vaegter HB, Finocchietti S, Handberg G, Arendt-Nielsen L. Assessment of musculoskeletal pain sensitivity and temporal summation by cuff pressure algometry: A reliability study. Pain 2015;156:2193-202.

36. Kosek E, Ekholm J, Hansson P. Modulation of pressure pain thresholds during and following isometric contraction in patients with fibromyalgia and in healthy controls. Pain 1996;64:415-423.

37. Cook DB, Stegner AJ, Ellingson LD. Exercise alters pain sensitivity in Gulf War veterans with chronic musculoskeletal pain. J Pain 2010;11:764-772.

38. Yang G, Baad-Hansen L, Wang K, Xie QF, Svensson P. A study on variability of quantitative sensory testing in healthy participants and painful temporomandibular disorder patients. Somatosens Mot Res 2014;31:62-71.

39. Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice AS. Reliability of conditioned pain modulation: a systematic review. Pain 2016;157:2410-2419.

40. Imai Y, Petersen KK, Morch CD, Arendt Nielsen L. Comparing test-retest reliability and magnitude of conditioned pain modulation using different combinations of test and conditioning stimuli. Somatosens Mot Res 2016;1-9.

Figure Legends

Fig. 1: Illustration of the experimental procedure performed on both testing days. Pressure pain thresholds (PPTs) were assessed on two assessment sites (quadriceps and trapezius) before and immediately after rest and exercise. 'IPAQ': International Physical Activity Questionnaire. 'PPT': Pressure pain threshold.

Fig. 2: Mean (+/- SEM) pressure pain threshold (PPT) recorded at two assessment sites (quadriceps and trapezius) before and immediately after 15 min quiet rest and 15 min bicycling. Significantly different compared with baseline (*, $P < 0.05$), significantly different compared with other assessment site (†, P < 0.05), and significantly different compared with rest condition ($, P < 0.05). 'Quad': m. quadriceps dominant side. 'Trap': upper trapezius muscle non-dominant side.

Fig. 3: Mean (+/- SEM, N = 34) exercise intensity over time performed by women (triangle) and men (square) [A], heart rate [B], rating of perceived exertion [C], and NRS scores of pain intensity [D] assessed during exercise in session 1 and session 2. Significantly different compared with other session (*, $P < 0.05$).

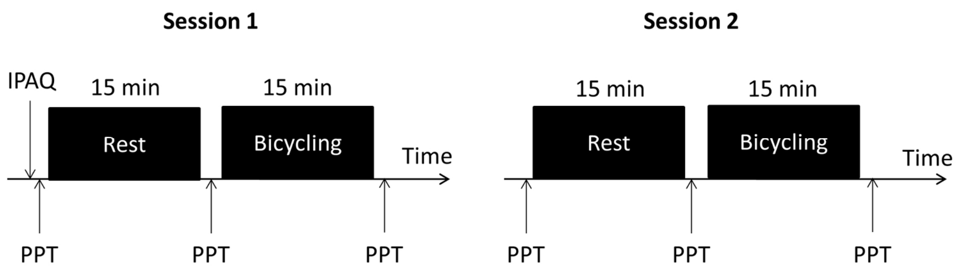

Illustration of the experimental procedure performed on both testing days. Pressure pain thresholds (PPTs) were assessed on two assessment sites (quadriceps and trapezius) before and immediately after rest and exercise. 'IPAQ': International Physical Activity Questionnaire. 'PPT': Pressure pain threshold.

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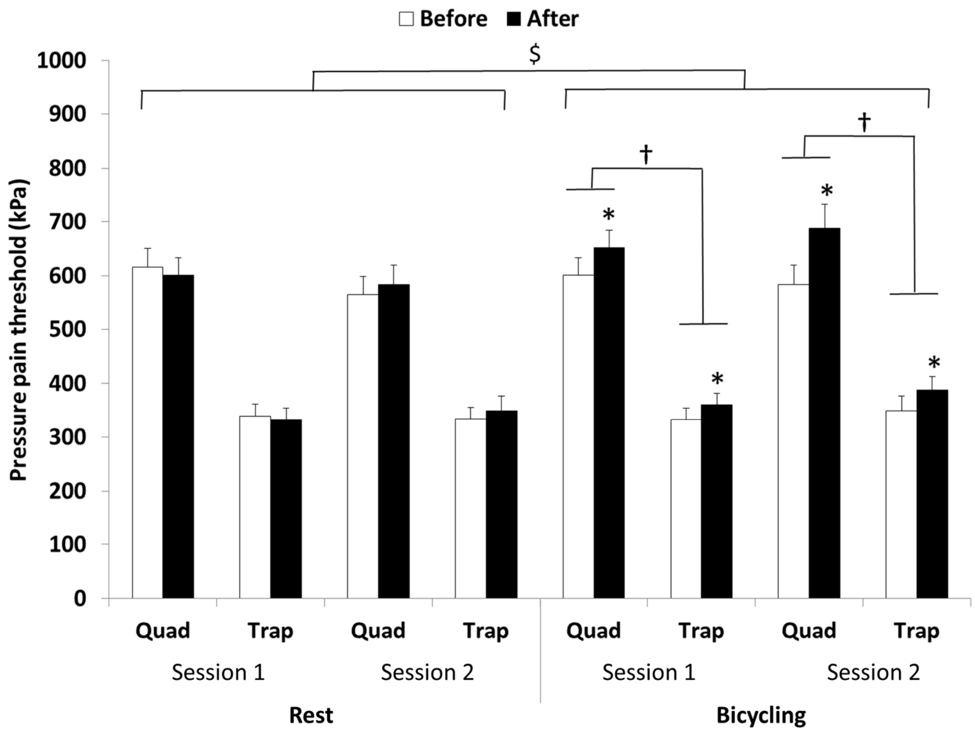

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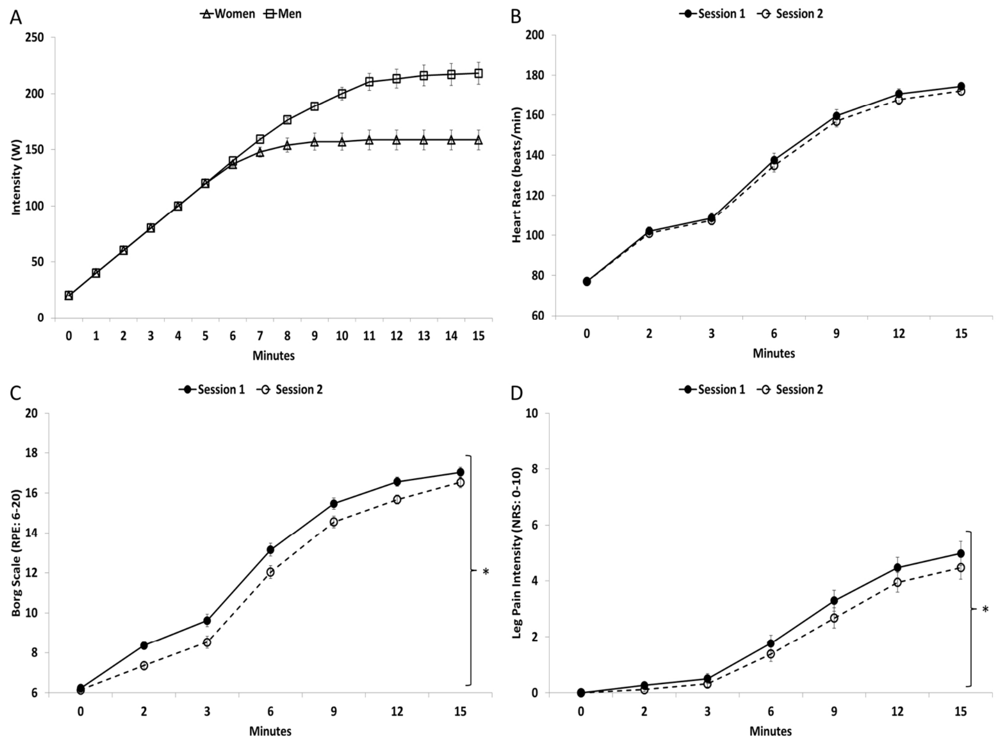

Mean (+/- SEM, N = 34) exercise intensity over time performed by women (triangle) and men (square) [A], heart rate [B], rating of perceived exertion [C], and NRS scores of pain intensity [D] assessed during exercise in session 1 and session 2. Significantly different compared with other session (*, P < 0.05).

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Table 1: Within-session test-retest reliability for pressure pain threshold at the dominant quadriceps and non-dominant upper trapezius muscles

Variable	Before Rest Mean ± SD (95%CI)	After Rest Mean ± SD (95%CI)	Absolute within-session difference Mean ± SD (95%CI)	Relative within-session difference Mean ± SD (95%CI)	P-value	Effect size	Pearson r	ICC3,1 (95%CI)	Standard error of measurement
Quad Session 1	616±198 kPa (547 - 685)	601±190 kPa (534 – 667)	15±99 kPa (-19 – 50)	1.6±15.9% (-3.9 – 7.1)	0.368	0.025	0.87 P<0.001	0.93 (0.86 – 0.97)	70 kPa
Quad Session 2	565±198 kPa (496 - 634)	583±213 kPa (509 – 658)	-18±98 kPa (-52 – 16)	-4.9±18.0% (-11.1 – 1.4)	0.277	0.036	0.89 P<0.001	0.94 (0.88 – 0.97)	70 kPa
Trap Session 1	340±127 kPa (295 - 384)	333±124 kPa (289 – 376)	7±54 kPa (-12 – 26)	0.7±15.7% (-4.7 – 6.2)	0.477	0.015	0.91 P<0.001	0.95 (0.90 – 0.98)	38 kPa
Trap Session 2	335±125 kPa (291 - 378)	350±154 kPa (297 – 404)	-15±59 kPa (-36 – 5)	-3.4±15.4% (-8.7 – 2.0)	0.137	0.066	0.93 P<0.001	0.95 (0.91 – 0.98)	42 kPa

Table 2: Between-session test-retest reliability for baseline pressure pain threshold (PPT) and exercise-induced hypoalgesia assessed at the dominant quadriceps and non-dominant upper trapezius muscles as absolute and relative change in PPT.

Variable	Session 1 Mean ± SD (95%CI)	Session 2 Mean ± SD (95%CI)	Absolute between-session difference Mean ± SD (95%CI)	Relative between-session difference Mean ± SD (95%CI)	P-value	Effect size	Pearson r	ICC3,1 (95%CI)	Standard error of measurement
Quad Baseline	616±198 kPa (547 - 685)	565±198 kPa (496 - 634)	51±148 kPa (-0.1 – 103)	7.1±24.0% (-1.3 – 15.5)	0.051	0.11	0.72 P<0.001	0.84 (0.68 – 0.92)	105 kPa
Trap Baseline	340±127 kPa (295 - 384)	335±125 kPa (291 - 378)	5±85 kPa (-25 – 35)	-1.5±24.9% (-10.2 – 7.2)	0.73	0.004	0.77 P<0.001	0.87 (0.74 – 0.94)	60 kPa
EIH Quad (absolute)	51±84 kPa (22 – 80)	104±117 kPa (63 – 145)	-53±121 kPa (-96 – -11)	-43.2±1216.1% (-467.5 – 381.1)	0.015	0.166	0.31 P=0.076	0.45 (-0.1 – 0.73)	86 kPa
EIH Quad (relative)	10.6±15.9% (5.1 – 16.1)	18.5±17.2% (12.5 – 24.5)	-7.9±20.3% (-15 – -0.8)	-9.1±1378.9% (-490.2 – 472.1)	0.03	0.135	0.15 P = 0.149	0.40 (-0.1 – 0.70)	14%
EIH Trap (absolute)	18±54 kPa (-1 – 36)	27±96 kPa (-6 – 61)	-10±92 kPa (-42 – 22)	2.3±216.3% (-73.2 – 77.7)	0.55	0.011	0.35 P = 0.042	0.46 (-0.1 – 0.73)	65 kPa
EIH Trap (relative)	7.3±17.6% (1.2 – 13.5)	12.7±27.3% (3.2 – 22.2)	-5.4±29.9% (-15.8 – 5.1)	0.6±245.1% (-84.9 – 86.1)	0.30	0.032	0.17 P = 0.335	0.27 (-0.4 – 0.64)	21%

Table 3: Crosstabulations of the EIH responders and non-responders at session 1 and session 2 at the quadriceps muscle [A] and the trapezius muscle [B], respectively.

A) EIH response in quadriceps muscle		**EIH response ≥ SEM in session 2**	
		Yes	**No**
EIH response ≥ SEM in session 1	**Yes**	11	5
	No	8	10

B) EIH response in trapezius muscle		**EIH response ≥ SEM in session 2**	
		Yes	**No**
EIH response ≥ SEM in session 1	**Yes**	5	9
	No	7	13